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ON THE CONDITIONS DETERMINING THE DISPOSITION OF THE CHROMATIC FILAMENTS AND CHROMOSOMES IN MITOSIS.

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Regarded from the purely physico-chemical or physiological standpoint protoplasm is essentially a complex aggregate of water and various colloidal and crystalloidal substances of which electrolytes form a chief part. It is also important to note that the *distribution* of the substances composing this aggregate is often, perhaps always, of a highly definite and specific kind; in recognition of this fact a more or less clearly defined "structure" has always been ascribed to protoplasm. Strictly speaking the term "structure" must include in its significance the distribution, grouping and relative positions of *all* the different cell-constituents; ordinarily, however, we do not regard the water and the more readily diffusible substances as distinctively structural elements; in general we mean by such elements those whose position in the complexus remains relatively constant and which give the form to the whole. On this definition we observe that it is primarily the *colloidal* substances of the cell that determine by their arrangement, distribution, and state of aggregation the particular type of structure that is presented; in other words, colloids form the basis of protoplasmic structure.

The reason for this is to be sought in a consideration of the general physico-chemical characteristics of this class of substances. The colloids of protoplasm, by virtue of their slow diffusibility, inability to penetrate one another, slow penetrability or virtual impenetrability to many crystalloids,¹ and relative unliability to chemical change, form throughout the cell a substratum which admits of the persistence within the cell-limits of a high degree of non-homogeneity. This condition is evidently indispensable

¹ Seen in the non-permeability of colloidal membranes to many dissolved substances.

for the existence of the localized differentiations that constitute organized structure. Its physiological counterpart is seen in the fact that there are constantly taking place within the single cell a multiplicity of diverse and often contrary, yet simultaneous processes; these normally are so coördinated that the entire system is enabled to maintain itself in equilibrium and to carry on its vital functions within a certain more or less limited range of conditions. Thus the possibility of this coördinated differentiation of structure and function, which is perhaps the most distinctive peculiarity of living matter, appears to depend on the above properties of the colloids and on the special manner in which these substances are disposed within the cell.¹

It is, therefore, to the conditions determining the disposition and state of aggregation of the colloids within the cell that we must look, when we attempt to explain the mode of origin of any given one of its structural features. Perhaps the most constant and distinctive of these features is the division into nucleus and cytoplasm. The proteids, which are the chief colloids of these two regions of the cell, are widely different in chemical nature; this implies a difference in their electrical properties, and with this is probably correlated the very typical segregation which they exhibit—the nuclear proteids forming a central aggregate which is almost always separated from the cytoplasmic proteids by a distinct membrane apparently impermeable to both. This seems the essential characteristic of the nucleated cell. A further peculiarity of the nuclear colloids is the remarkably definite disposition which they show at times; this is especially marked at the period of cell-division, when they form arrangements and go through movements of so characteristic a kind that the name *karyokinesis* has been given to the entire process. It is this peculiar mode of distribution of the nuclear colloids that I propose briefly to consider in the present paper: the movements and grouping of the nuclear colloidal aggregates, chromatic filaments and chromosomes, though apparently complex, are,

¹ For two important recent discussions of the part which colloids play in protoplasm, cf.: Hofmeister, "Die chemische Organisation der Zelle," Braunschweig, Vieweg, 1901; and Pauli, "Der kolloidale Zustand und die Vorgänge in der lebendigen Substanz," Braunschweig, Vieweg, 1902.

I believe, due essentially to a series of relatively simple conditions the nature of which I shall attempt to analyze below.

What then are the conditions that determine the disposition adopted by the chromosomes and the chromatic filament in the dividing cell? In the prophase of mitosis the chromatin¹ becomes disposed in so many cells in the form of one or more elongated deeply staining² filaments coiled within the nuclear membrane into a roughly spiral form, that the arrangement is regarded as typical. This is the well-known "spireme" stage. The spireme then segments into the definite number of chromosomes characteristic of the cell; the nuclear membrane disappears and the chromosomes are drawn into the equatorial plane of the cell; here they assume the arrangement known as the "equatorial plate" in which, it is to be noted, a distinct interval separates each chromosome from its neighbors. Each then divides, typically by a process of longitudinal splitting, into the two daughter-chromosomes which recede toward opposite poles of the cell; here later the daughter-nuclei are reconstituted from the two groups of daughter-chromosomes thus formed.

Both the adoption of this remarkable spiral arrangement and the manner in which the chromosomes become disposed in the equatorial plate are, I shall attempt to show, dependent mainly upon one simple physical property which the particles of chromatin possess in common with other similarly charged colloidal particles — namely, the property of mutual repulsion. It may now be considered as finally established that a colloidal substance in solution is in the condition of a more or less finely divided suspension, each particle of which is at a different electrical potential from the adjoining layer of liquid, *i. e.*, carries an electri-

¹ This is the cytological term; chemically this substance is undoubtedly nucleoprotein in nature; the chromosomes are probably similar to sperm-nuclei in their composition, as we may infer from the similarity between the micro-chemical reactions of the two. The nuclein of sperm-heads is relatively simple in composition and contains a large proportion of nucleic acid. For a general account see R. Burian in Asher u. Spiro's *Ergebnisse der Physiologie*, 3, 1894, I. Abtheilung, *Biochemie*, pp. 48-106.

² The increased depth of stain is an indication of increased acidity, *i. e.*, increased liberation of hydrogen ions; this involves an increase in the electrical negativity of the colloidal substance and consequently in the force with which adjacent portions of the filament repel one another.

cal charge. The sign of the charge is positive in the case of basic, negative in the case of acid bodies, since the former liberate negative (OH^-) ions, the latter positive (H^+) ions; the colloid particles themselves are thus left with free positive or negative charges respectively. All particles in a given colloidal solution, having like charges, must for this reason repel one another. Apparently the stability of a colloidal system depends upon this mutual repulsion of the particles of solute, since neutralization of the charge, as by the action of ions of opposite sign, results in an aggregation of the particles and the removal of the colloid from solution; to this action is to be ascribed the precipitating effect of ions.

If the particles composing the chromatic filaments and the chromosomes similarly repel one another—as in accordance with their colloid nature they must do—mutual repulsion will play an important part in determining the disposition which these structures adopt within the cell. The chromatin being an acid body must liberate hydrogen ions and become negatively charged, and this to a greater degree the higher its proportion of nucleic acid. I have elsewhere pointed out that sperm-nuclei, which are rich in nucleic acid, show a particularly strong tendency to travel with the negative stream when an electric current is passed through a solution containing them; this fact may be regarded as confirmatory of the view that the chromosomes, which in composition are probably very similar to sperm-nuclei, are likewise negatively charged bodies.¹

Again the prevailingly central position of the nucleus within the cell—especially the cell about to undergo mitosis—indicates that there exists within the cell an influence tending to draw the nuclear colloids toward a central position. There are reasons for regarding this influence also as electrical in its nature; it seems highly probable that in the dividing cell the intra-nuclear colloids and the cytoplasmic colloids are opposite in their electrical properties²; they are certainly contrasted in their general chemical

¹ R. S. Lillie, *American Journal of Physiology*, 8, 1903, p. 273. Also *BIOL. BULL.*, 4, 1903, p. 175.

² Proteids are amphoteric bodies, *i. e.*, may liberate a preponderance either of hydrogen or of hydroxyl ions according to conditions.

behavior ; at all events they react toward acid and basic staining reagents in precisely opposite manners. This would indicate that the prevalent cytoplasmic colloids are preponderatingly¹ basic in character, *i. e.*, that their particles liberate hydroxyl ions chiefly, and are accordingly for the most part positively charged. It is conceivable that the hydrogen ions from the nuclear proteids and the hydroxyl ions from the cytoplasmic proteids, both of which ions have high migration velocities, find their way throughout the entire cell and unite with each other to form water. The colloids themselves, on the other hand, though of opposite sign, are unable so to unite, or can unite only partially, on account of the impossibility of their diffusing through one another ; they are therefore left in the cell with free and opposite charges. Possibly a partial union of the nuclear and the cytoplasmic colloids may take place at the boundary between the two aggregates ; it is known that colloids of opposite electrical sign will precipitate each other when their solutions are mixed, presumably by each effecting a neutralization of the other's charge.² Now it is a very remarkable fact that the nucleo-proteids of the cell are almost invariably separated from the cytoplasmic proteids by a well defined membrane, the " nuclear membrane," which is very possibly a precipitation-membrane formed at the surface of contact between the two oppositely charged colloidal aggregates. If this is so, it becomes intelligible why further neutralization of the charges is prevented and how each set of colloids is enabled to retain its charge in the presence of the other. The condition thus reached is that of a negatively charged aggregate, *viz.* : the nucleus with its contained chromatin, in the midst of a positively charged field consisting of the numerous positively charged colloidal particles of the cytoplasm.³

The central position of the chromatin is now easily explained as due to the attraction which the aggregate of oppositely charged cytoplasmic colloids exercise upon it, the resultant effect of all

¹ Cf. R. S. Lillie, *loc. cit.*

² Biltz, *Berichte der deutschen chemischen Gesellschaft*, 37, 1904, p. 1095.

³ This suggests J. J. Thomson's conception of the atom as a system of negative electrons moving in a field of uniform positive electrification. It would be strange if such a parallel should exist between the respective units of living and of lifeless matter.

these attractive forces being to draw the chromatin toward the center of the cell. On the other hand adjacent chromosomes or adjacent portions of the chromatic filament are by their own mutually repellent action exposed to an opposite influence which resists their approach toward one another. Hence the position of equilibrium, *i. e.*, the stationary position which they finally adopt, must be one where these two opposed sets of influences exactly balance each other.

The formation of the spireme and of the equatorial plate may then be ascribed to these two chief conditions, one the attractive influence that draws the chromatin toward a central position and tends to keep it there, the other the mutually repellent action of the chromatin particles themselves. The stationary position is one of equilibrium between these two opposed tendencies.

I should add here, in reference to the striking fact that the chromosomes at the metaphase occupy positions side by side *in a single plane*—that of the equator of the cell—that this remarkable peculiarity of disposition also remains to be accounted for. As yet, however, I know of no experimental facts bearing directly on this problem; and in the following explanation of the manner in which the equatorial plate is formed, I shall assume from the first that the chromosomes are free to move only in this single plane, without as yet attempting to explain why this is so.

EXPERIMENTAL.

If the conditions of spireme-formation and of equatorial plate-formation are similar to those suggested above, it should be possible to simulate these phenomena experimentally by making use of artificial filaments composed of mutually repellent units arranged in rows, and by subjecting these to the action of some centrally attracting force. The disposition and relative positions assumed by such filaments should, if the above hypothesis is correct, resemble those exhibited by the chromatic filament and chromosomes in the dividing cell.

In pursuance of this idea I have experimented with filaments consisting of rows of floating magnetized needles. The experiments of Alfred Mayer on floating magnets are well known to physicists; he studied many years ago the behavior of groups

of small magnetized needles which were floated by being passed through small discs of cork and placed on the surface of water in such a manner that all the magnets were vertical in position and similarly oriented, *i. e.*, with all the north (or south) poles uppermost. Such floating needles repel one another with a force inversely proportional to the square of their distance apart. If then over such a group floating with (for example) north poles uppermost a large bar-magnet is suspended in a vertical position with its south pole downward, all the needles are, by the horizontal resultant of the attractive force of the large magnet, drawn in toward a central position immediately below the latter; here they adopt arrangements of perfectly definite and regular configuration, whose exact form varies with the number of needles but shows great constancy for any given number.¹ The condition of stability of such systems is that the mutual repulsion which the small magnets exercise upon one another shall be exactly balanced by the centripetal attractive force due to the large magnet.

In their manner of grouping such floating magnets exhibit a close resemblance to the chromosomes of many equatorial plates, for example, those of the sea-urchin egg (seen in face). So far as I am aware, however, no attempt has hitherto been made to explain the grouping of chromosomes as due to conditions of the above kind. The following experiments have been designed with a view to testing this hypothesis.

To simulate the nuclear chromatic filament, the following procedure is employed: Small, similarly oriented, magnetized needles are strung at short and regular intervals (say 6 millimetres apart) along a delicate silk thread, preferably a single silk filament; each needle is passed through a small cubical piece of cork; the entire filament can then be floated on the surface of water with the needles vertical in position. When left undisturbed such a filament tends to be pulled out into a straight line by the mutual repulsion of its units; this form would be taken if the filament were ideally flexible and the supporting fluid devoid of viscosity, since then the average distance of the needles apart would be as

¹ Figures showing these arrangements will be found in any good text-book of physics, for example Ganot's.

great as possible under the conditions. Actually, however, the straight form is only approximated.

Such a filament may be caused to assume the spireme form as follows: A large bar magnet is suspended vertically over the filament with its north pole next the projecting south poles of the needles (or *vice versa*) and at a suitable distance from the latter. It is then found that the filament is drawn together by the attraction of the magnet into a more or less regular, close coil or spireme-like form, remarkably like that shown by the nuclear filament in the prophase of mitosis. Briefly, the explanation of this behavior is as follows: The filament is, by the attraction of the large magnet, confined within a limited space

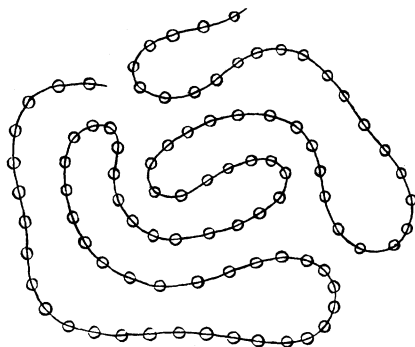


FIG. 1. Artificial spireme from a single filament; the small circles indicate the exact number and relative positions of the magnets.

just as the chromatic filament in the cell is confined by the nuclear membrane. Now, since mutual repulsion tends to prevent approximation of adjacent portions of the filament, the latter is forced so to dispose itself that, while occupying the greatest space possible under the conditions, the average distance between its adjacent portions is also as great as possible. This however leads inevitably to the production of the characteristic coiled or roughly spiral form which is the only one that satisfies these conditions, that is, in which the entire system is in equilibrium.

Figs. 1 and 2 are reproductions of exact drawings showing the form of the artificial spireme in each of two experiments, one with a single filament, the other with six separate filaments.

Their resemblance to the nuclear spiremes is evident. Such spireme figures may exhibit a great variety of forms; at present, however, I shall not attempt to enumerate these in detail; a later paper will contain drawings showing the exact form assumed by

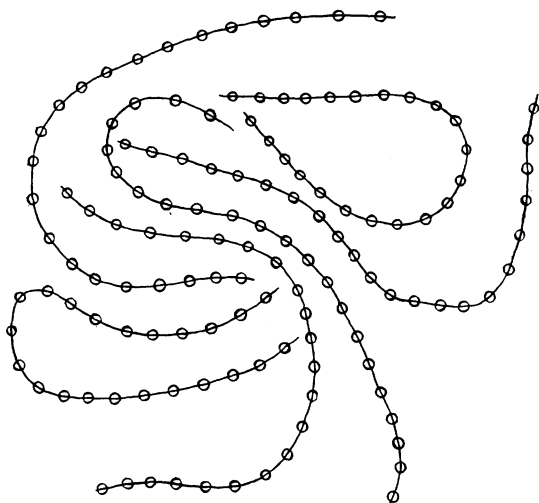


FIG. 2. Spireme from six separate filaments and disposition of individual magnets shown by the small circles.

the filaments under a variety of conditions: varying their number, size and structure, as well as the intensity of the central attractive force.

The formation of the equatorial plate may be simulated by the use of similar floating aggregates of needles, which in this case

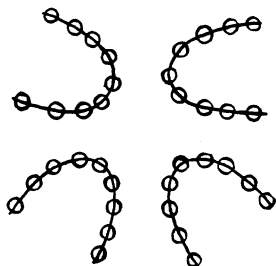


FIG. 3. Arrangement shown by group of four chromosome models each with 9 magnets.

are strung along flexible wires capable of being bent into any desired shape. In this way the aggregate may be given the

form of any one of the various types of chromosome, and it thus becomes possible to study the effect which changing the form of the chromosomes has upon the configuration of the equatorial

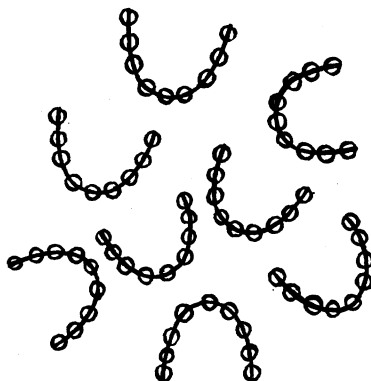


FIG. 4. Arrangement shown by eight loop-shaped chromosome models.

plate. These chromosome models repel one another, and, like the isolated needles, they are found to exhibit very constant and regular configurations when subjected, as above described, to the attractive influence of the large magnet. The resemblance to

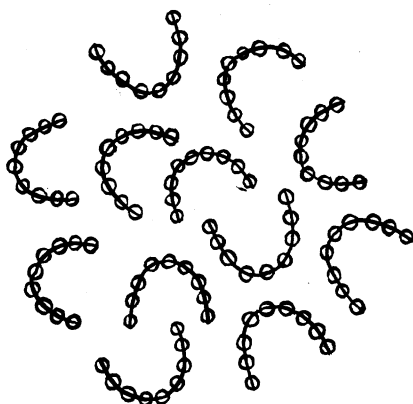


FIG. 5. Group of twelve loop-shaped chromosome models.

the configurations observed in the equatorial plates of actual cells is often very striking. I have made experiments with varying numbers of such chromosome-like aggregates and have

attempted to determine what the possible and stable configurations—that is, the conditions of equilibrium—are for any given number of chromosomes up to twelve.

Figures 3, 4 and 5 give exact representations of configurations shown respectively by groups of four, eight and twelve simple loop-shaped chromosome models. It should be added that the number of possible configurations becomes very great when the number of chromosomes is large. In the full paper drawings of a number of these will be given. I shall then consider the condition of their formation in greater detail.

It is my intention to investigate further the possibilities of this type of magnetic model. It is possible that such phenomena as the aggregation of the chromosomes in a single plane, their longitudinal splitting, and the separation of the daughter-chromosomes, may be simulated by means of such models. Much light may thus be thrown upon the mechanics of these characteristic phenomena.

It is to be noted that in experiments of the above type the filaments and chromosomes are arbitrarily confined in their movements to a single plane, that of the surface of the water. In this respect such models are incomplete, since, in the cell, movement is possible in all three dimensions. It seems clear, however, that, setting aside this partial and incidental limitation, these imitations of cell-phenomena are often surprisingly true to nature. *In so far as regards the mode of disposition* of the chromatic filaments and of the rows of magnets, the observed resemblance may be held to imply an identity in the essential determining conditions. These conditions are those of mutual attractions and repulsions, which are common to both systems. In other respects, it need scarcely be said, the two systems are widely different. Still, so long as science proceeds, as it has always done, by the progressive identification of apparently widely different things it will be necessary to isolate by analysis the features in which otherwise diverse phenomena agree, and to disregard, for the time being, their differences. We may with justification infer from the above that attractions and repulsions—here undoubtedly electrostatic in nature, since colloid bodies are concerned—play a most important part in determining the disposi-

tion of certain of the structural elements of the cell. How far such conditions have to do with form-determination in general is a further question, and one that stands in urgent need of thorough-going investigation. It may prove necessary to add electrostatic attractions and repulsions to the list of the conditions determinative of organic form and structure.

NAPLES, December 12, 1904.